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Salivary Gland Involvement in Chronic Graft-Versus-Host Disease: Prevalence, Clinical Significance, and Recommendations for Evaluation

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Although xerostomia is a commonly reported complaint in patients with chronic graft-versus-host disease (cGVHD), criteria for evaluating the prevalence and characteristics of salivary gland involvement have not been well defined in this patient population. Previous studies also have made no distinction between salivary and mucosal oral cGVHD. We systematically evaluated signs and symptoms of sicca in a large cohort of patients with cGVHD (n = 101) using instruments widely used to study Sjogren's syndrome. Xerostomia was reported by 77% of the patients and was associated with xerophthalmia in all but 1 case. The salivary flow rate was ≤0.2 mL/min in 27%, and ≤0.1 mL/min in 16%. Histopathological changes, consisting of mononuclear infiltration and/or fibrosis/atrophy, were present in all patients with salivary dysfunction. Importantly, there was no correlation of salivary and oral mucosal involvement in cGVHD. Patients with cGVHD-associated salivary gland involvement had diminished oral cavity-specific quality of life and lower body mass index. Salivary gland involvement is a common and clinically distinct manifestation of cGVHD. Formal evaluation of salivary function using standardized criteria is needed, and this could be incorporated as an outcome measure in clinical trials of cGVHD.

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INTRODUCTION

Chronic graft-versus-host disease (cGVHD) is the single most important complication in long-term survivors after allogeneic hematopoietic stem cell transplantation (allo-HSCT) [1]. Involvement of cGVHD of the salivary and lacrimal glands results in Sjogren's syndrome (SS)-like manifestations, including hyposecretion of saliva and tears.

Xerostomia can be a distressing symptom, and decreased salivary flow may lead to reduced food intake, dental caries, and oral mucosal infection with *Candida* species [2]. In addition, salivary dysfunction may be associated with other, more severe manifestations of cGVHD, such as pulmonary involvement [3]. Therefore, formal assessment of salivary gland dysfunction is important in an overall assessment of cGVHD. Salivary gland function can be assessed using noninvasive tests, and could be used as an outcome in clinical trials.

Whereas the prevalence of xerophthalmia after allo-HSCT has been estimated to range between 40% and 70% in various studies [4-6], the prevalence of salivary gland involvement has not been well described [7]. Oral dryness complaints in cGVHD are often reported as "oral" or "mouth" involvement, and are not distinguished from oral mucosal lesions [8]. This is in part because older literature implied that the pathologic changes of the minor salivary glands found in cGVHD represented a continuum of the oral mucosal lesions found in the disease. However, oral mucosal and salivary gland involvement in cGVHD bear close resemblance

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Table 1. Patient Characteristics (n = 101)

Diagnosis, n (%)	
Acute leukemia/myelodysplastic syndrome	42 (41)
Chronic leukemia	23 (23)
Lymphoma	21 (21)
Multiple myeloma	8 (8)
Aplastic anemia/myelofibrosis	5 (5)
Paroxysmal nocturnal hemoglobinuria	2 (2)
Conditioning, n (%)	
Myeloablative	59 (59)
Reduced intensity	42 (41)
Donor type, n (%)	
Matched sibling donor	73 (72)
Matched unrelated donor	28 (28)
Stem cell source, n (%)	
Bone marrow	19 (19)
Peripheral blood	80 (79)
Unknown	2 (2)
Karnofsky Performance Status score, mean (SD), range	81 (12), 30-100
Months since transplantation, mean (SD), range	43 (38), 4-201
Months since cGVHD diagnosis, mean (SD), range	36 (37), 1-196

cGVHD indicates chronic graft-versus-host disease.

to autoimmune disorders affecting these tissues, specifically oral lichen planus and SS, which occur independently of each other and affect distinct patient populations. Although guidelines for evaluation of lacrimal dysfunction in cGVHD were defined in the recent National Institutes of Health (NIH) consensus criteria [9,10], no recommendations have been provided for evaluation of salivary gland involvement. Therefore, the aim of this study was to systematically examine the characteristics and correlates of salivary gland function in cGVHD and to determine whether oral mucosal and salivary gland pathology in cGVHD occurred independently. We also developed preliminary guidelines that could be used to evaluate patients with cGVHD who report oral dryness.

PATIENTS AND METHODS

Patients

A total of 101 consecutive adult patients enrolled in a cGVHD cross-sectional study (clinicaltrials.gov #NCT00331968) were included in this study. The study was approved by the National Cancer Institute's Institutional Review Board, and informed consent was obtained from all participants. Patients underwent comprehensive multispecialty clinical evaluation, laboratory testing, and research sample collection, and they completed a series of patient-reported outcome measures. The diagnosis of cGVHD was established using NIH consensus criteria [9]. The sample had a mean patient age of 45.9 ± 12 years (range, 20-66 years), predominantly white (n = 92; 91%), and with approximately equal representation of males (n = 53; 52%) and females (n = 48; 48%). The median time from transplantation to cGVHD diagnosis was 5 months (range, 3-35 months). Most patients had active moderate or severe cGVHD requiring continuation of systemic immunosuppression. Transplant and cGVHD characteristics for the sample are presented in Tables 1–3.

Table 2. Characteristics of Patients with cGVHD (n = 101)

	Salivary Dysfunction	
	Present (n = 22)	Absent (n = 59)
Onset of cGVHD, n (%)		
De novo	10 (45)	21 (36)
Quiescent	2 (9)	13 (22)
Progressive	10 (45)	25 (42)
Platelet count ≤100,000, n (%)	5 (23)	4 (7)
Clinician global rating of cGVHD, n (%)		
Mild	0 (0)	2 (3)
Moderate	8 (36)	24 (41)
Severe	11 (50)	26 (44)
Missing	3 (14)	7 (12)
Change in cGVHD over previous month, n (%)		
Better	5 (23)	10 (17)
About the same	9 (41)	18 (31)
Worse	8 (36)	31 (53)
Intensity of current immunosuppression, n (%)		
None	3 (14)	5 (8)
Mild*	2 (9)	6 (10)
Moderate†	9 (41)	16 (27)
High‡	8 (36)	22 (59)
Chronic GVHD clinical severity score, mean (SD), range	37 (10), 19-56	30 (9), 7-48
Lee cGVHD symptom scale total score, mean (SD), range	34 (14), 11-69	25 (13), 1-60

cGVHD indicates chronic graft-versus-host disease.
*Single agent prednisone <0.5 mg/kg/day.
†Single agent prednisone ≥0.5 mg/kg/day or single agent/modality with and without prednisone ≥0.5 mg/kg/day.
‡Two or more agents/modalities with and without prednisone ≥0.5 mg/kg/day.

Clinical Evaluations

Salivary and lacrimal symptoms were evaluated using methods described by the American-European Consensus Group (AECG) for SS [11]. The presence of xerostomia and xerophthalmia were determined using the 3-item AECG screening questionnaire, which assesses both the presence and duration of symptoms. Patient-reported symptoms of dry mouth have been reported to not correlate well with objective measures of salivary function [12]. In addition, xerostomia severity was graded on a patient-reported scale of 0-10.

Evaluation of salivary gland function was performed by measuring the unstimulated salivary flow rate using 5-minute saliva collection into a preweighed 50-ml centrifuge tube in a modification of a procedure described previously [13]. Objective evaluation of lacrimal function included Schirmer's test (performed with local anesthesia) and evaluation of keratopathy and conjunctival involvement by fluorescein and lissamine green staining according to European-U.S. criteria for evaluation of SS [11]. A Schirmer's test score of ≤5 mm in 5 minutes was considered abnormal.

Table 3. Prevalence of cGVHD Organ System Involvement Based on NIH Scoring

	Salivary Dysfunction	
Organ System Involved, n (%)	Present (n = 19)	Absent (n = 52)
Skin	13 (68)	41 (79)
Oral cavity (any manifestation)	18 (95)	37 (71)
Eyes	18 (95)	40 (77)
Lungs	10 (53)	26 (51)
Liver	9 (47)	25 (48)
GI tract	11 (58)	17 (33)
Joints/fascia	8 (42)	29 (56)
Genital tract (women only)	7 (50)	13 (41)
Total number of organ systems involved, median (range)	4.5 (3-7)	4.0 (2-8)

cGVHD indicates chronic graft-versus-host disease.

Oral mucosal changes were evaluated using a scale developed for scoring oral mucositis lesions and oral lichen planus, a condition clinically very similar to oral cGVHD [14].

Oral cavity-specific quality of life (ie, the impact of an oral health condition on talking, eating, self-esteem, mood, and role function) was assessed with the 14-item Oral Health Impact Profile (OHIP-14) questionnaire, which has been validated in various oral conditions [15]. Health-related quality of life (HRQL) was assessed using the Functional Assessment of Cancer Therapy (FACT-G) questionnaire [16], and selected aspects of cGVHD were evaluated on the Lee cGVHD symptom scale [17]. Overall and organ-specific cGVHD severity was assessed based on detailed multidisciplinary clinical and diagnostic evaluation of the skin, mouth, eyes, liver, lungs, gastrointestinal (GI) system, functional capacity, and gynecologic manifestations in women. Scoring of individual organ systems was based on the NIH criteria for diagnosis and staging [9]. Using objective criteria, organ system subspecialists graded the severity of cGVHD in each of 8 organ systems, and a summed severity score ranging from 0-100 was calculated.

Histopathology

Minor salivary glands (MSGs) were collected from the lower lip of consenting patients using a standard procedure [18], fixed in formalin, and embedded in paraffin. Hemtoxylin and eosin-stained sections were evaluated by a pathologist with expertise in cGVHD and SS who was blinded to clinical evaluation data. The degree of mononuclear infiltration was determined based on focus score (number of lymphocytic foci of $\geq$50 cells per 1 mm^2 of the minor salivary gland section) and Greenspan grade (0-4 scoring of the infiltrate density) [19]. Glandular atrophy and fibrosis were scored separately on a scale of 0-3, based on the proportion, by thirds, of glandular parenchyma affected. The presence or absence of perilobular fibrosis, duct rupture, and ductitis was recorded as well.

Statistical Methods

The χ^2 test or Fisher's exact test was used to evaluate differences in proportions as appropriate. Group differences were assessed using Wilcoxon's rank-sum test for continuous variables and the exact Cochran-Armitage test for ordered and categorical variables. Analysis of covariance was used to identify the strength of the association between outcomes of interest while controlling for the effects of other parameters that might have an impact on the findings. Correlations between 2 continuously measured parameters were determined using Spearman's rank correlation coefficient, r, with the strength of the association between parameters interpreted as follows: $|r| > 0.70$, strong correlation; $0.50 < |r| < 0.70$, moderately strong correlation; $0.30 < |r| < 0.50$, weak to moderately strong correlation; and $|r| < 0.30$, weak correlation.

All P values are 2-tailed and are presented without formal adjustment for multiple comparisons. However, in view of the exploratory nature of the study, the varying degrees of independence among the tests, and the number of tests performed, a P value $<.005$ might be considered a statistically significant result, whereas a result for which $.005 < P < .05$ would be considered a strong trend. All calculations were performed using MedCalc version 9.3.0.0 (Mariakerke, Belgium) and SPSS for Windows version 15.0 (SPSS Inc, Chicago, IL).

RESULTS

Prevalence of Salivary and Lacrimal Dysfunction in cGVHD Patients

A summary of clinical parameters is presented in Supplemental Table 1. Seventy-eight patients (77%) completed the 6-item SS questionnaire. Overall, 66 patients (86%) gave at least one positive answer on the questionnaire, with 60 (77%) reporting oral complaints and 51 (67%) reporting ocular complaints. There was a significant association between symptoms of ocular and oral dryness, with 46 patients (59%) reporting both complaints ($P = .0017$; χ^2 for number of patients with ocular or oral complaints).

We then evaluated lacrimal and salivary function using clinician-assessed measures. Based on the NIH consensus criteria on cGVHD diagnosis and staging [9,10], we considered an abnormal Schirmer tear test and/or the presence of keratopathy in at least one eye to indicate decreased lacrimal function. Decreased salivary gland function was defined as an unstimulated salivary flow rate of <1 mL in 5 minutes (0.2 mL/min), a value consistent with the lower limit of normal salivary function determined by population studies [20].

Using these criteria, salivary dysfunction was less prevalent than lacrimal dysfunction in our cGVHD

cohort (27% [22/81] vs 82% [80 of 98]). Seventy-two of the 98 patients for whom results were available had a Schirmer tear test score of ≤5 mm (73%). Keratopathy as established by positive corneal staining, was seen in 57 of these patients (58%). As expected, the severity of keratopathy was correlated with Schirmer tear test values ($r = -0.52$; $P < .001$). All but one patient with salivary dysfunction had lacrimal dysfunction as well.

We also examined the correlation of objective measures of salivary and lacrimal flow and patients' symptoms. There was a moderately good correlation between self-reported xerostomia and salivary flow rate ($r = -0.51$; $P < .001$). A similar correlation was found between patient-reported ocular dryness and Schirmer test results ($r = -0.55$ for the left eye and -0.52 for the right eye; $P < .001$).

An easily administered screening test would be useful to guide subsequent evaluation of lacrimal and salivary gland function in cGVHD. Consequently, we evaluated the predictive ability of the SS questionnaire (SSQ) with regard to decreased salivary and/or lacrimal function on clinical testing. Seventy-six patients had both patient-reported and clinician-assessed evaluations of salivary and lacrimal function. Fifty-four out of 64 patients with lacrimal dysfunction and 5 out of 12 patients with a normal eye exam endorsed at least 1 of the 3 ocular items on the SSQ (sensitivity, 84%; specificity, 58%; positive predictive value [PPV], 92%; negative predictive value, [NPV] 41%). Of 21 patients with a low salivary flow rate, 19 responded positively to at least one item on SSQ. Among 55 patients with clinically normal salivary flow rates, 23 had a negative questionnaire (sensitivity, 90%; specificity, 42%; PPV, 37%; NPV, 92%). These data suggest that the eye component of the SSQ might be most useful to rule in patients likely to have positive findings on the ophthalmologic exam, whereas the oral component would be most useful for ruling out significant salivary flow reduction. The predictive values reported here should be interpreted with caution, however, given that the prevalence of lacrimal and salivary gland dysfunction in our study might be higher than that in the general cGVHD/allo-HSCT population.

Lack of Association between Salivary Gland Dysfunction and Oral Mucosal Disease

To explore the hypothesis that salivary gland and mucosal involvement represent distinct manifestations of cGVHD, we evaluated the covariation between the presence and severity of mucosal involvement and salivary gland dysfunction in our cohort. Of 75 patients for whom both data points were available for analysis, only 14 had both salivary gland dysfunction and oral mucosal involvement. Forty-one patients had only mucosal dysfunction, and 5 had only salivary gland dysfunction. Fifteen patients had neither mucosal involvement nor salivary dysfunction ($P = 1.0$, Fisher's exact test for proportions of patients with mucosal and/or salivary involvement). There was no correlation between the degree of mucosal changes as measured by specific grading scale and salivary flow rate ($r = 0.09$; $P = .42$). Similarly, there was no difference in cGVHD oral mucosal severity scores between patients with (median, 4.5; 25th-75th percentile, 0.25-6.0; n = 19) and without salivary gland dysfunction (median, 3.5; 25th-75th percentile, 0-9.8; n = 56; $P = 0.93$) or in salivary flow rates between patients with (median, 0.36 mL/min; 25th-75th percentile, 0.20-0.67 mL/min; n = 55) and without mucosal disease (median, 0.35 mL/min; 25th-75th percentile, 0.20-0.59 mL/min; n = 20; $P = .59$). These findings suggest that salivary and oral mucosal involvement in cGVHD are distinct disease manifestations and not extensions of one another.

Salivary Gland Dysfunction Is Associated with Impaired Oral Cavity-Specific Quality of Life and Decreased Body Mass Index

We used a validated oral cavity-specific quality-of-life questionnaire (OHIP-14) to evaluate the impact of salivary gland dysfunction on the oral quality of life of patients with cGVHD. OHIP-14 scores were significantly higher (indicating greater impairment in quality of life) in patients with salivary gland dysfunction (median, 12; 25th-75th percentile, 10-29; n = 21 vs median, 6.5, 25th-75th percentile, 1-13; n = 54; $P < .001$). Greater impairment in oral cavity-specific quality of life also was associated with more perceived oral dryness ($r = 0.49$; $P < .001$) and lower salivary flow rate ($r = -0.41$; $P < .001$). Surprisingly, we found no statistically significant difference in OHIP-14 score between patients with and those without oral mucosal cGVHD (median, 10; 25th-75th percentile, 3-17; n = 51 vs median, 6.5, 25th-75th percentile, 1-10; n = 20; $P = .19$). Furthermore, although quality-of-life scores correlated well with the degree of oral discomfort ($r = 0.43$; $P < .001$), they were not associated with the clinician-assessed severity of oral mucosal disease ($r = 0.21$; $P = .09$). Although other oral conditions, such as caries or periodontal disease, could have had an impact on oral quality-of-life scores, such changes were not often seen in our patient cohort.

There was no statistically significant relationship between the presence of salivary gland dysfunction and overall HRQL as assessed by FACT-G, a global HRQL scale, controlling for age and cGVHD severity ($P = .193$). Salivary dysfunction can result in impaired nutritional status, however. Patients with salivary gland dysfunction reported significantly greater difficulty in swallowing solid foods ($P = .001$) on the Lee cGVHD

symptom scale. After adjusting for cGVHD severity, patients with salivary involvement also had significantly ($P = .008$) lower body mass index (BMI) than those with an intact salivary flow rate (estimated marginal mean BMI, 21.9; 95% CI, 19.6-24.2; n = 22 vs 25.3; 95% CI, 24.1-26.6; n = 59). These differences in BMI remained when we adjusted specifically for the severity of cGVHD GI manifestations ($P = .001$). There was no difference in BMI between groups with and without GI symptoms (mean BMI, 24.2; 95% CI, 20.8-27.6; n = 19 vs 25.1; 95% CI, 24.0-26.2; n = 79; $P = .51$).

Histopathological Changes in Patients with Salivary Gland Dysfunction

MSG tissue specimens were available from 36 patients. Six patients did not have complete clinical information and thus were excluded from analysis. Gross examination commonly revealed such features as periductal infiltration (Figure 1A), atrophy of salivary gland lobules, and periglandular fibrosis (Figure 1B). In some patients, atrophy was so severe that no MSG tissue could be identified.

Patients with clinically observed salivary gland dysfunction had higher atrophy (median, 2; 25th-75th percentile, 0.5-3.0; n = 8 vs 0.5; 25th-75th percentile, 0-2.0; n = 22; $P = .16$), fibrosis (median, 2; 25th-75th percentile, 0.5-3.0; n = 8 vs 0; 25th-75th percentile, 0-2.0; n = 22; $P = .12$), and focus scores (median, 0.50; 25th-75th percentile, 0-2.5; n = 8 vs 0; 25th-75th percentile, 0-1.0; n = 22), although the differences did not reach statistical significance. There was a difference in Greenspan grade (median, 2.5; 25th-75th percentile, 2.0-4.0; n = 8 vs 2; 25th-75th percentile, 1.0-3.0; n = 22; $P = .04$). The group of patients with a Greenspan grade >1 had lower salivary flow rates (median, 0.24 mL/min; 25th-75th percentile, 0.16-0.43 mL/min; n = 21 vs 0.51 mL/min; 25th-75th percentile, 0.38-0.64 mL/min; n = 9; $P = .02$). This group also included only patients with salivary gland dysfunction as defined by our flow rate criterion, whereas all patients without salivary gland dysfunction had a Greenspan grade ≤1.

Currently, there is no agreement on how to evaluate and report MSG involvement based on histopathological grading. We propose a composite histopathological grading score of Greenspan grade and degree of fibrosis as a tool to evaluate the overall involvement of MSGs. Greenspan grade reflects the degree of infiltration in the glands, and fibrosis and atrophy are indicative of long-term damage. In this subject cohort, atrophy was highly correlated with fibrosis ($r = 0.96$; $P < .001$) and thus would not have added information to the overall score. Mild infiltration and minor degrees of fibrosis are nonspecific and become more prevalent with aging [21]. Indeed, in our sample, age was correlated with degree of the glandular fibrosis ($r = 0.59$; $P = .005$). Thus, we propose a cutoff of ≥3 for the composite score to indicate likely involvement with cGVHD.

If a composite MSG score of ≥3 is used as an indicator of salivary gland cGVHD, then 100% of patients with a salivary flow rate ≤0.2 mL/min will have histopathological evidence of MSG involvement. However, 60% of patients with histological evidence of MSG disease in our sample had a normal salivary flow rate.

Factors Associated with Salivary Dysfunction in cGVHD

To better understand the factors contributing to salivary dysfunction in cGVHD, we evaluated the association of various demographic and transplantation-related variables with the presence of salivary gland dysfunction. In a multivariate model, none of the demographic and transplant parameters examined, including age, intensity and type of the conditioning regimen (myeloablative vs nonmyeloablative, use of total body irradiation), type of donor (matched related vs unrelated or haploidentical), severity of oral mucosal cGVHD, or time after diagnosis of cGVHD was predictive of salivary gland dysfunction (data not shown).

Salivary gland involvement is commonly observed in patients with scleroderma, and decreased salivary gland function has been linked to the glandular fibrosis in these patients [22]. Because sclerodermatous skin involvement is the hallmark of cGVHD, we hypothesized that skin sclerosis in cGVHD may be associated with salivary gland involvement. We found no association between sclerotic skin involvement (superficial or deep) and salivary dysfunction in our cohort ($P = .81$, Fisher's exact test). There were no differences in mean salivary flow rate or degree of salivary gland fibrosis between patients with and without sclerotic skin involvement (data not shown).

Saliva contains numerous factors important for innate and adaptive mucosal immunity including secretory IgA. Salivary dysfunction might predispose to oral colonization and subsequent recurrent pulmonary infections [23]. Furthermore, sicca symptoms have been reported in association with pulmonary involvement in cGVHD [3]. Using NIH cGVHD lung scores (a score that ranges from 0 to 3), we observed a trend toward an association between salivary gland dysfunction and greater pulmonary cGVHD involvement (n = 81; $P = .015$, Cochran-Armitage exact test).

Laboratory Parameters in cGVHD Patients with Sicca Syndrome

Autoantibodies are commonly associated with and are used for diagnostic purposes in many autoimmune conditions. Antibodies to nuclear antigens Ro (SSA) and La (SSB) are found in a high percentage of patients with SS [11]. Although we detected a wide range of

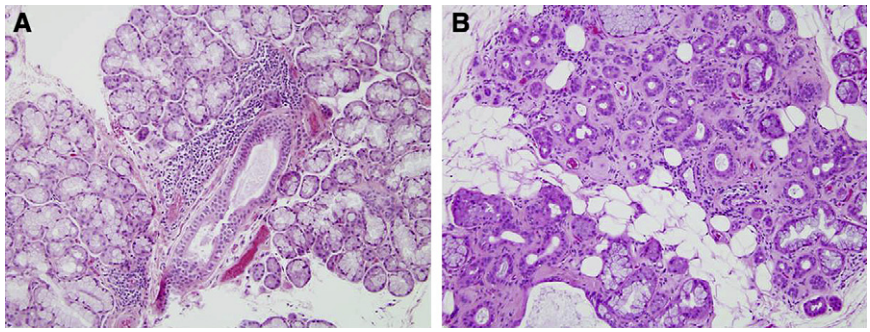

Figure 1. (A) Photomicrograph of a salivary gland showing SS-like periductal inflammation, with a periductal infiltrate composed predominantly of lymphocytes. (Hematoxylin and eosin; original magnification 200×.) (B) Severe atrophy with interstitial fibrosis. Most of the mucinous acinar parenchyma has been lost from this lobule, replaced by ductular metaplasia and increased perilobular adipose tissue. There is a mild, predominant lymphocytic infiltrate in the fibrous interstitium. (Hematoxylin and eosin; original magnification 200×.)

autoantibodies in our patient cohort, the individual prevalence was very low. For example, only 4 patients had a positive extractable nuclear antigen screen, a test for all intranuclear antigens used in rheumatology that includes Ro/SSA and La/SSB (Supplemental Table 2).

DISCUSSION

This is the first study to date to comprehensively analyze clinical and pathological features of sicca syndrome in cGVHD using tools that are widely used to study lacrimal and salivary gland dysfunction in SS. We found a very high prevalence of sicca complaints in patients with cGVHD, as well as a significant association between complaints of xerophthalmia and xerostomia. Salivary and lacrimal glands share a common developmental origin, and it is possible that similar or identical antigens in both organs are recognized by alloreactive T cells. However, it also is possible that our estimate of prevalence exceeds that in the general cGVHD population because of a referral bias, because the overwhelming majority of patients in our study had moderate or severe cGVHD.

In the prospective studies of cGVHD, oral cavity is the second most commonly involved area [5]. Whether the symptoms observed result from mucosal (ie, lichen planus-like) disease or salivary gland involvement is unclear, however. In fact, in older [7,24] and more recent [25] studies dealing with the diagnosis and staging of oral cGVHD, mucosal and MSG biopsy are advocated as equivalent diagnostic tests. Although criteria for clinician-assessed lacrimal gland involvement have been defined by the recent NIH consensus guidelines, salivary and mucosal involvement were grouped as "oral" involvement [9,10]. But such a grouping of mucosal and salivary gland involvement may be counterproductive, because salivary gland disease and mucosal diseases are managed differently. For example, whereas topical anti-inflammatory agents are commonly used for superficial mucosal inflammation, topical therapy is unlikely to be effective for salivary gland involvement. Patients with significant decreases in salivary gland output often benefit from cholinergic agonists, such as pilocarpine.

Our data provide the first evidence of an important distinction between salivary and oral mucosal involvement in cGVHD, as demonstrated by the lack of correlation between symptoms and signs related to each subset. This is demonstrated by the lack of difference in salivary flow rate between patients with and without mucosal disease, as well as the lack of correlation between severity of oral mucosal disease as assessed by standardized grading and flow rate measurements. Indeed, patients with mucosal involvement had higher mean flow rates. The major clinical significance of salivary gland dysfunction is demonstrated by the greatly decreased oral cavity-specific quality of life in patients with xerostomia. In fact, salivary dysfunction had a much greater impact on oral cavity-specific quality-of-life score than did mucosal disease. Whereas there was no significant association between salivary dysfunction and overall HRQL when cGVHD severity and age were controlled for, the importance of salivary function for nutritional status is underscored by lower BMI values and significantly greater reported difficulty swallowing solid foods in patients with salivary involvement. Our results suggest that salivary gland dysfunction might partially explain the observed association between cGVHD and weight loss [26].

Because no guidelines exist for assessing salivary gland function in cGVHD, we adapted methods recommended for evaluating salivary function in SS [11]. We propose using the SSQ as an initial screening test of salivary dysfunction, which can be performed at each visit in the context of screening for cGVHD. In addition, quantitative evaluation of xerostomia on a scale of 0-10 should be performed in all patients. In patients with a positive response to the questionnaire, the unstimulated salivary flow rate can be measured as an objective evaluation of salivary gland function. This test does not involve any special equipment apart from a preweighed or graduated plastic tube, is reliable, and can be easily performed by nonspecialists with minimal

training. We chose a cutoff of 0.2 cc/min as the lower limit of normal based on the flow rates in a large cohort of normal volunteers [20]. Whereas the 0.1 cc/min recommended by the SS consensus criteria is very specific for salivary gland involvement by SS, it is not sufficiently sensitive for use in the cGVHD population and will miss many patients with early disease.

We propose a composite score of mononuclear infiltration and fibrosis as a histopathological criterion of MSG involvement with cGVHD. Fibrosis and lymphocytic infiltration are included in 2 other scales developed to evaluate cGVHD in MSGs, but these scales are limited quantitatively, or the scores are based on combined mucosal and salivary changes [27-29]. In our cohort, all patients with a salivary flow rate <0.2 cc/min had positive findings on biopsy (MSG score ≥ 3). Although this finding needs to be validated in a prospective study, such patients probably do not need an MSG biopsy to confirm salivary gland involvement. However, MSG biopsy, as well as testing of salivary gland functional reserve by measuring stimulated salivary flow rate, might be useful in identifying those patients most likely to benefit from treatment. In our opinion, patients late in the disease process, as manifested by the extensive destruction of MSG tissues with fibrosis and atrophy, are probably unlikely to regain significant function. Conversely, patients with lymphocytic infiltration but minimal MSG destruction are probably the most likely to benefit from immunosuppressive treatment.

The significance of positive histopathology as a "gold standard" for salivary gland involvement is unclear, particularly in asymptomatic patients. Whereas positive findings are probably sufficient evidence for the alloreactive response to salivary gland tissue, whether such involvement would necessarily progress to symptomatic disease is not known. Similarly, it is not known whether treatment would reverse the salivary gland pathology, and at what point such treatment would be most effective. In this sense, using objective parameters of salivary function could be useful to define patients who might benefit most from treatment and who are at greatest risk for xerostomia-related complications. These hypotheses could be tested in the future therapeutic trials and natural history studies.

Although the clinical manifestations of lacrimal and salivary gland injury are similar in chronic cGVHD and SS, significant differences exist. For example, the prevalence of autoantibodies in our study was very low overall, and autoantibodies associated with the SS (anti-SSA) were detected in only one patient. Similarly, the pattern of salivary gland histology is different in SS, with the much more pronounced lymphocytic infiltration leads to a higher focus scores [19]. This could be explained by the different pathogenetic mechanisms involved, which merits further study at the cellular and molecular levels.

Sicca syndrome is a common manifestation in patients with scleroderma, and pathological changes in the salivary glands have been proposed as part of the generalized fibrotic process in scleroderma [22]. Because sclerodermatous skin involvement is a common feature of cGVHD, we evaluated the hypothesis that these 2 manifestations could be linked. Although fibrosis is a prominent histopathological feature of salivary gland disease, we found no association between skin sclerosis and sicca syndrome, suggesting that skin and salivary/lacrimal glands are independently affected in cGVHD.

Our study found no association between the salivary gland dysfunction in cGVHD and various patient and transplant characteristics, including age and intensity of the conditioning regimen. Although some previous studies have linked total body irradiation to subsequent sicca manifestations [30,31], our study did not confirm such an association. Indication of more significantly impaired pulmonary function in patients with salivary gland dysfunction is intriguing and merits further study. At least one study reported an association between sicca syndrome and pulmonary involvement after allo-HSCT [3]. Unfortunately, however, no precise definition of sicca syndrome was given, complicating interpretation of the results.

There are several limitations to this study. Our cohort included primarily patients with long-standing and more severe disease, with a mean disease duration of about 3 years. The data collected provide a cross-sectional representation of a condition with very diverse and complex manifestations. The findings presented may not be directly applicable to routine care of a general cGVHD patient population. Nevertheless, we believe that this work lays the foundation for a comprehensive assessment of the salivary component of cGVHD in the research setting. Further refinements to the proposed guidelines must be made in the context of prospective studies and controlled clinical trials.

In conclusion, salivary gland involvement by cGVHD contributes significantly to morbidity and impaired quality of life in this patient population. Our results demonstrate the distinction between salivary and oral mucosal involvement, and we have proposed specific criteria for assessing salivary gland involvement in cGVHD. Prospective studies are needed to validate our findings and address issues of the optimal timing and nature of therapeutic intervention to prevent and ameliorate the loss of salivary gland function in cGVHD.

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SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbmt.2010.03.023

REFERENCES

1. Baird K, Pavletic SZ. Chronic graft-versus-host disease. *Curr Opin Hematol*. 2006;13:426-435.
2. Mathews SA, Kurien BT, Scofield RH. Oral manifestations of Sjogren's syndrome. *J Dent Res*. 2008;87:308-318.
3. Sakaida E, Nakaseko C, Harima A, et al. Late-onset noninfectious pulmonary complications after allogeneic stem cell transplantation are significantly associated with chronic graft-versus-host disease and with the graft-versus-leukemia effect. *Blood*. 2003;102:4236-4242.
4. Tichelli A, Duell T, Weiss M, et al. Late-onset keratoconjunctivitis sicca syndrome after bone marrow transplantation: incidence and risk factors. European Group for Blood and Marrow Transplantation (EBMT) Working Party on Late Effects. *Bone Marrow Transplant*. 1996;17:1105-1111.
5. Flowers ME, Parker PM, Johnston LJ, et al. Comparison of chronic graft-versus-host disease after transplantation of peripheral blood stem cells versus bone marrow in allogeneic recipients: long-term follow-up of a randomized trial. *Blood*. 2002;100:415-419.
6. Mohty M, Kuentz M, Michallet M, et al. Chronic graft-versus-host disease after allogeneic blood stem cell transplantation: long-term results of a randomized study. *Blood*. 2002;100:3128-3134.
7. Schubert MM, Sullivan KM. Recognition, incidence, and management of oral graft-versus-host disease. *NCI Monogr*. 1990;135-143.
8. Lee SJ, Vogelsang G, Flowers ME. Chronic graft-versus-host disease. *Biol Blood Marrow Transplant*. 2003;9:215-233.
9. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease, I: diagnosis and staging working group report. *Biol Blood Marrow Transplant*. 2005;11:945-956.
10. Pavletic SZ, Martin P, Lee SJ, et al. Measuring therapeutic response in chronic graft-versus-host disease. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease IV: Response Criteria Working Group report. *Biol Blood Marrow Transplant*. 2006;12:252-266.
11. Vitali C, Bombardieri S, Jonsson R, et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis*. 2002;61:554-558.
12. Fox PC, Busch KA, Baum BJ. Subjective reports of xerostomia and objective measures of salivary gland performance. *J Am Dent Assoc*. 1987;115:581-584.
13. Navazesh M, Christensen C, Brightman V. Clinical criteria for the diagnosis of salivary gland hypofunction. *J Dent Res*. 1992;71:1363-1369.
14. Piboonniyom SO, Treister N, Pitiphat W, et al. Scoring system for monitoring oral lichenoid lesions: a preliminary study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2005;99:696-703.
15. Hegarty AM, McGrath C, Hodgson TA, et al. Patient-centered outcome measures in oral medicine: are they valid and reliable? *Int J Oral Maxillofac Surg*. 2002;31:670-674.
16. Cella DF, Tulsky DS, Gray G, et al. The Functional Assessment of Cancer Therapy scale: development and validation of the general measure. *J Clin Oncol*. 1993;11:570-579.
17. Lee S, Cook EF, Soiffer R, et al. Development and validation of a scale to measure symptoms of chronic graft-versus-host disease. *Biol Blood Marrow Transplant*. 2002;8:444-452.
18. Stewart CM, Bhattacharyya I, Berg K, et al. Labial salivary gland biopsies in Sjogren's syndrome: still the gold standard? *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2008;106:392-402.
19. Greenspan JS, Daniels TE, Talal N, et al. The histopathology of Sjogren's syndrome in labial salivary gland biopsies. *Oral Surg Oral Med Oral Pathol*. 1974;37:217-229.
20. Sreebny LM, Valdini A. Xerostomia, part I: relationship to other oral symptoms and salivary gland hypofunction. *Oral Surg Oral Med Oral Pathol*. 1988;66:451-458.
21. Drummond JR, Chisholm DM. A qualitative and quantitative study of the ageing human labial salivary glands. *Arch Oral Biol*. 1984;29:151-155.
22. Avouac J, Sordet C, Depinay C, et al. Systemic sclerosis-associated Sjogren's syndrome and relationship to the limited cutaneous subtype: results of a prospective study of sicca syndrome in 133 consecutive patients. *Arthritis Rheum*. 2006;54:2243-2249.
23. Taylor DC, Cripps AW, Clancy RL. A possible role for lysozyme in determining acute exacerbation in chronic bronchitis. *Clin Exp Immunol*. 1995;102:406-416.
24. Loughran TP Jr., Sullivan K, Morton T, et al. Value of day 100 screening studies for predicting the development of chronic graft-versus-host disease after allogeneic bone marrow transplantation. *Blood*. 1990;76:228-234.
25. Shulman HM, Kleiner D, Lee SJ, et al. Histopathologic diagnosis of chronic graft-versus-host disease: National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease II: Pathology Working Group report. *Biol Blood Marrow Transplant*. 2006;12:31-47.
26. Jacobsohn DA, Margolis J, Doherty J, et al. Weight loss and malnutrition in patients with chronic graft-versus-host disease. *Bone Marrow Transplant*. 2002;29:231-236.
27. Sale GE, Shulman HM, Schubert MM, et al. Oral and ophthalmic pathology of graft-versus-host disease in man: predictive value of the lip biopsy. *Hum Pathol*. 1981;12:1022-1030.
28. Horn TD, Rest EB, Mirenski Y, et al. The significance of oral mucosal and salivary gland pathology after allogeneic bone marrow transplantation. *Arch Dermatol*. 1995;131:964-965.
29. Hiroki A, Nakamura S, Shinohara M, et al. A comparison of glandular involvement between chronic graft-versus-host disease and Sjogren's syndrome. *Int J Oral Maxillofac Surg*. 1996;25:298-307.
30. Calissendorff B, el Azazi M, Lonnqvist B. Dry eye syndrome in long-term follow-up of bone marrow-transplanted patients. *Bone Marrow Transplant*. 1989;4:675-678.
31. Chaushu G, Itzkovitz-Chaushu S, Yefenof E, et al. A longitudinal follow-up of salivary secretion in bone marrow transplant patients. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 1995;79:164-169.